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Morphological Identification of Helminth Parasites in *Gallus gallus domesticus* in Lucknow, U.P., India

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ABSTRACT

Poultry farming is one of the most developed, profitable, and intensively practiced forms of animal production. Gastrointestinal worms are well-known parasites of poultry birds responsible for decreasing poultry productivity. So the microscopic study revealed the possibility of differentiating helminthic parasites. Cestodes and nematodes were collected from the domestic fowl (*Gallus gallus domesticus*) in Lucknow, Uttar Pradesh. Light microscopy was used for the detailed identification of helminths. In total, 300 gastrointestinal tracts were inspected for helminths between July 2023 and June 2024. The helminths that were gathered were identified using Yamaguti's (1961) and Soulsby's (1982) keys and descriptions. In Lucknow, a significant percentage of helminth infections (95%) were found in household poultry. Two cestode species (*Raillietina tetragona* & *Cotugnia diagnopora*) and one nematode species (*Ascaridia galli*) were found throughout the investigation. The majority of the helminths were retrieved from the small intestine, followed by the caecum. According to this study, helminths cause major issues for domestic chickens which reduce productivity. So suitable control measures on the basis of study must be developed to improve output.

INTRODUCTION

A worldwide problem that affects both native chicken species and poultry productivity is parasitic infection in chickens (Permin *et al.,* 2020; Shifaw *et al.,* 2021). The majority of chicken production area is non-intensive and primarily farmed on mixed-species farms in backyards, gardens, and orchards. Important health problems in mixed-species farming are often related to the outdoor access area, exposing the animals to various viral, bacterial and parasitic infections some of which may only influence the animals' own welfare, whereas others may also endanger the health of conventional livestock (Kijlstra *et al.,* 2006). Though parasites can infect chickens through polluted feed, water, or livestock equipment (Parede *et al.,* 2005). These parasites have the potential to modify the host's immune response to other illnesses and have immunomodulatory effects result in altered chicken development, egg production, meat quality, and even cause premature death of the host (Daş *et al.,* 2021; Soulsby *et al.,* 1982). Parasitic diseases are one of the obstacles that confront large-scale poultry production. There are numerous endoparasites that might cause significant damage to the organs they inhabit (Sheikh et al., 2016).

Inflammation and bleeding in the intestinal epithelium of the gastrointestinal tract can result from endoparasitic infections (Urquhart *et al.,* 1996). The most widespread and common parasites in poultry industry are thought to be nematodes, trematodes, and cestodes (Poulsen *et al.,* 2000). These helminths disrupt the host's metabolism, leading to poor feed utilization and reduced growth and production (Gauly *et al.,* 2007). The nutrients required for native chicken growth will be absorbed by these worm parasites in their bodies (Sudaryani *et al.,* 2007). Helminths with microscopic thread-like cylindrical bodies, hooks, and a hard body cuticle are well-adapted for long-term survival in their hosts (Soulsby *et al.,* 1982). The study may provide important information for identifying the helminths mentioned here, assisting in the development of effective control measures found in domestic chickens (*Gallus gallus domesticus*) in Lucknow, U.P., India.

Study area
The study was carried out at an altitude of approximately 404 feet above sea level in and around Lucknow. The total area of the Lucknow district is 2,528 square kilometers. Lucknow is situated on the northwest shore of the Gomti River and is bordered to the east by Barabanki, to the west by Unnao, to the south by Raebareli, and to the north by Sitapur. The climate of this city is humid subtropical, with hot, dry summers with thunderstorms from late March to June and chilly, dry winters from mid-November to February. From July to September, the city receives an average of 896.2 millimeters (35.28 inches) of rain from the south-west monsoon winds. In January, there are sporadic frontal rainstorms. The lowest temperature in the winter is between 3 °C (37 °F) and 7 °C (45 °F), while the highest is around 25 °C (77 °F).

Study Population
Domestic chickens that were kept in backyard environments are part of the study population. The age of the chicken was recorded as reported by owners.

Study period
The study was conducted in Lucknow District, Uttar Pradesh, from July 2023 to June 2024 in order to ascertain the specifics of domestic poultry intestinal parasites.

Parasitological examination
A total of 300 gastrointestinal tracts (GITs) of domestic poultry were gathered from five study sites locations between July 2023 and June 2024. The GI tracts were knotted at both ends and put in sterile bags with labels to prevent internal material leakage. The GI tracts were taken to the Parasitology Laboratory of the Department of Zoology at Babasaheb Bhimrao Ambedkar University in Lucknow for a parasitological investigations and a thorough dissection to look for parasites according to the protocol as illustrated by Cable *et al.*, 1958.

Preservation
Helminths were removed from the host using forceps, washed with salted water, killed in hot 70% ethanol, and preserved in the 70% ethanol.

Light Microscopy (LM)
Helminths were removed by brush and fine forceps and washed in normal saline. Nematode were taken out from the host using forceps, washed with salted water. Live parasites were passed through hot 70% alcohol till death, stored in the glycerine alcohol solution and thick parasites were kept in lectophenol untill get transparent, mounted them in DPX . Parasites were observed under light bright field microscope. Cestodes were fixed in Cornoy's fixative for 24 hours, removed the fixative by washing or rehydration with ascending alcohol grades , stained with Borax carmine, dehydrate with descending grades of alcohol, transferred to xylene for clearing before mounting them in DPX (Dextrine Plasticized Xylene). Parasites were identified according to the keys and description given by Yamaguti *et al.*, 1961 and Soulsby *et al.*, 1982.

RESULTS

Morphology Identifying Characteristics of Helminths Thorough investigation of intestinal tract and collected all worms from all 300 samples, three helminths were confirmed. Those were one nematode (*A. galli*) species and two cestodes (*R. tetragona and C. diagnopora*) species.

Light Microscopy of Ascaridia galli

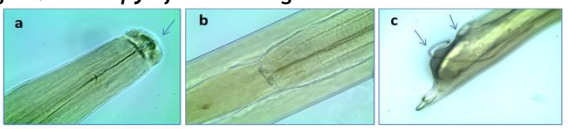

Figure 1: Ascaridia species (a) Anterior oral opening surrounded by three prominent lips (arrow) (b) filariform esophagus with simple cuticular structure (c) Posterior end showing preanal sucker (arrow) immediately anterior to spicules (arrow)

Morphological characteristic of Ascaridia galli
Adult worms were extracted from the small intestines; they were semitransparent and yellowish white in appearance. Males ranged in length from 45 to 80 mm. Three prominent lips encircled the oral aperture. On the dorsal lip and each of the subventral lips, there were two distinct papillae (Fig.1a). The intestine was simple and the esophagus was filariform, with the entire body covered in a hard, proteinaceous layer known as cuticle. Cuticular alae (flap like projection) were underdeveloped in anal region and the cuticle was clearly striated (Fig.1b). Male ascarids with ventrally coiled tails and precloacal suckers exhibited morphological sexual dimorphism. The precloacal or preanal sucker was located just in front of the spicules, and the anus was located toward the posterior end. A sclerotized ring which serves as an attachment aid during copulation supported the precloacal sucker. Two well-developed, uneven spicules were present at the posterior end of the worms (Fig.1c).

Light Microscopy of Raillietina tetragona

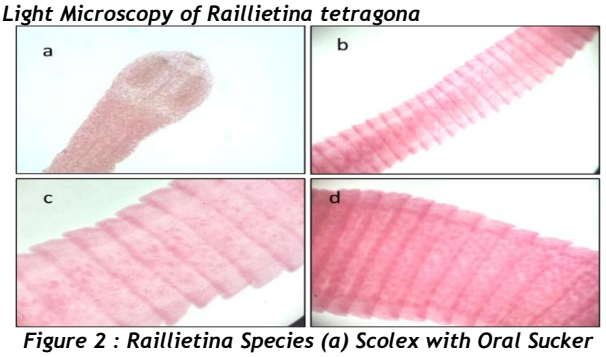

Figure 2 : Raillietina Species (a) Scolex with Oral Sucker and rostellum (b) Immature segments (c) Mature segments with fully developed reproductive organs (d) gravid segments filled with eggs.

Morphological characteristic of *Raillietina tetragona*
Several fully developed *Raillietina tetragona* , each 15 to 30 cm long, were extracted from the small intestine.. These cestodes species were elongated, dorso-ventrally flattened, and white in appearance. The body was separated into the "scolex" (head region), "neck" (unsegmented), and "strobila" (highly segmented body), (Fig. 2a). Scolex is a tiny, spherical organ. The apical rounded rostellum of each scolex is medium in size and equipped with numerous tiny hammer-shaped hooks grouped in a single row. Additionally, four ovoid suckers with many rows of spines surround the scolex (Fig. 2a). Proglottids, a group of body segments that progressively grew in size posteriorly, made up the strobila. The common genital pores were solitary and located in front of the anterior one-third of the lateral margin of the mature segment, which was longer than broad. Male and female reproductive organs were present in each adult

proglottid (Fig. 2c). The testes were situated behind the vitellarium and on either side of the ovary.

Light Microscopy of *Cotugnia digonopora*

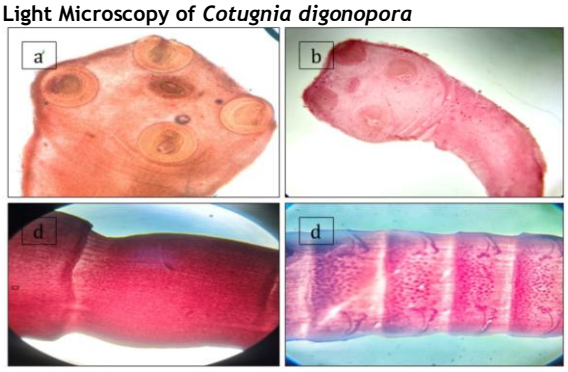

Figure 3 : *Cotugnia* Species (a) Scolex showing four unarmed suckers and rostellum (b) Scolex with neck region without defined segments (c) Mature segments wider than length, and have two sets of reproductive organs showing testes with 90-100 in number, ovary V-shaped. (d) Gravid segments filled with eggs and genital hole is medium in size and oval in shape, and the ovary is V-shaped on both sides of the segment.

Morphological characteristic of *Cotugnia digonopora*

Several fully grown *Cotugnia digonopora*, measuring 15-35 cm long were separated from the gastro intestinal tract. These cestodes were lengthy, dorso-ventrally flattened, and had a white hue. The scolex, or head area, was separated from the rest of the body, the extensively segmented body known as the "strobila" appears after an unsegmented "neck" (Fig. 2a). Each scolex had a rounded apex. with two rows of small hooks on the rostellum, which is medium and unarmed. Four ovoid suckers were also present around the scolex which was also unarmed (Fig. 3a). The proglottids, which made up the strobila, were a group of body parts that progressively grew in size. The common genital pores were solitary and located in front of the anterior third of the mature segment's lateral border; the mature segment was longer than wide. A pair of male and female reproductive organs was present in every adult proglottid (Fig. 2 c & d). Testes were found on each side of the segment's V-shaped ovary, and the genital opening was oval in shape and medium in size.

There is inadequate morphological evidence available in Lucknow (U.P.) to accurately identify helminths in backyard chickens. The identification of helminths relied only on gross appearance and host preference sites. Therefore, extensive observations of morphology were undertaken in this work to provide a foundation in order to detect helminths in backyard chickens. A nematode species and two cestodes species were identified i.e., *Ascaridia galli* (Fig.1) *Raillietina tetragona* & *Cotugnia diagnopora* (Fig. 2 & 3). The nematode (male) *A galli* species was identified as yellowish white and semitransparent. The species ranged in length from 45 to 80 mm. Three large lips surrounded the oral opening. There were two distinct papillae on the dorsal lip and one on each of the subventral lips. The gut was simple, and the esophagus was filariform. The cuticle, a thick proteinaceous coat, covered the whole body. The cuticular alae were undeveloped, and the cuticle was clearly striated. The anus at the back and the precloacal or preanal sucker just in front of the spicules were the two most noticeable openings. At the posterior end of the worms, there were two well-developed unequal spicules. These characteristic were supported with the previously reported characteristics of *A galli* (Duggal *et al.*, 2000; Magwisha *et al*. 2002 & Basrat *et al.*,2014). Between these two species, *Raillietina* has a cosmopolitan distribution affecting domestic birds including chickens (Dakhil and Al-Musaedi 2022). Around 75 percent of helminth infection are due to different species of *Raillietina* and considered as most prevalent genus around the world (Shifaw *et al.*, 2021). As an essential component of the chicken's diet, their intermediate hosts, beetles and ants are more widely distributed. It is reasonable to presume that the birds may have acquired helminth infection as a result of their diet. In the current study the key physical traits of Raillietina and Cotugnia, including as the form of the sucker, the arrangement of hooks in the rostellum, and the location of the genital pore and reproductive organs, were used to identify the species. The recognized morphological characteristic of *Raillietina tetragona* showed features like scolex, neck and strobila. Additionally, four ovoid suckers with many rows of spines surrounding the scolex. The common genital pores were located in front of the anterior third of the mature segment's lateral border, and the mature segment was longer than wide. A pair of male and female reproductive organs was present in each fully grown proglottid. The ovary has testes behind the vitellarium and on both sides. These characteristic were supported with the previously reported characteristics of *Raillietina tetragona* (Butbooncho *et al.*, 2016; Roy *et al.*, 2012). *R. tetragona* was discovered as a whitish, extremely elongated, dorso-ventrally flattened cestode isolated from a fowl small intestine. The detailed morphological properties of these cestodes were identical to the findings of many authors (Soulsby, 1982; Ramadan and Zanda, 1991; Ahmed and Nabila, 2004). In this study the general morphology of *Cotugnia diagnopora* showed features like extended and flattened dorso-ventrally. Scolex was separated from the segmented body by the unsegmented neck. Rostellum with apical rounded with two rows of tiny hooks. Four ovoid, unarmed suckers ringed the scolex as well. The proglottids, a group of body parts that progressively grew in size posteriorly, made up the strobila. The typical genital openings were solitary and located forward of the anterior third of the mature segment's border on the side, and the mature segment was longer than wider. A double pair of male and female reproductive organs was present in every adult proglottid. Testes were located on both sides of the V-shaped ovary medium-sized, oval-shaped genital pores on either side of the segment. These characteristic were also supported with the previously reported characteristics of Cotugnia diagnopora (Pasquale et al., 1890 & Siddiqui et al.,1961)

DISCUSSION

This study addresses the occurrence and morphology of gastrointestinal helminths in domestic chickens in various localities of Lucknow. Helminths were found in abundance (95% frequency). The characteristics found through light microscopy shed light on the incidence and morphological identification of helminth parasitic infections in poultry. Most of the chickens in this study had harbored multi-species helminth infections, indicating that the environment and the structure of the poultry rearing system are very conducive to the spread and persistence of helminth parasites. Additional large scale researches are needed to assess the helminth infection effect on domestic chickens health and productivity.

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